

Supporting Information

Metabolomics and genomics evidence for compromised bile acid homeostasis by senecionine, a hepatotoxic pyrrolizidine alkaloid

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Figure S1 Quantitative ^1H NMR spectrum of senecionine. Senecionine (MW 365) and 1, 4-dinitrobenzene (internal standard, MW 168, purity 99.5%) were dissolved in 1.0 ml of CDCl_3 and analyzed on Bruker Make Avance-400 operating at 400.13 MHz (9.38 T) for proton equipped with a 5 mm multinuclear broad band observe (BBO) probe head. All data was acquired and processed using Bruker's Topspin 3.0 software. The signal at 6.23 Hz was for H-2 in senecionine while the signal at 8.45 Hz was for the four hydrogen in 1, 4-dinitrobenzene. The purity of senecionine was determined as 92%.

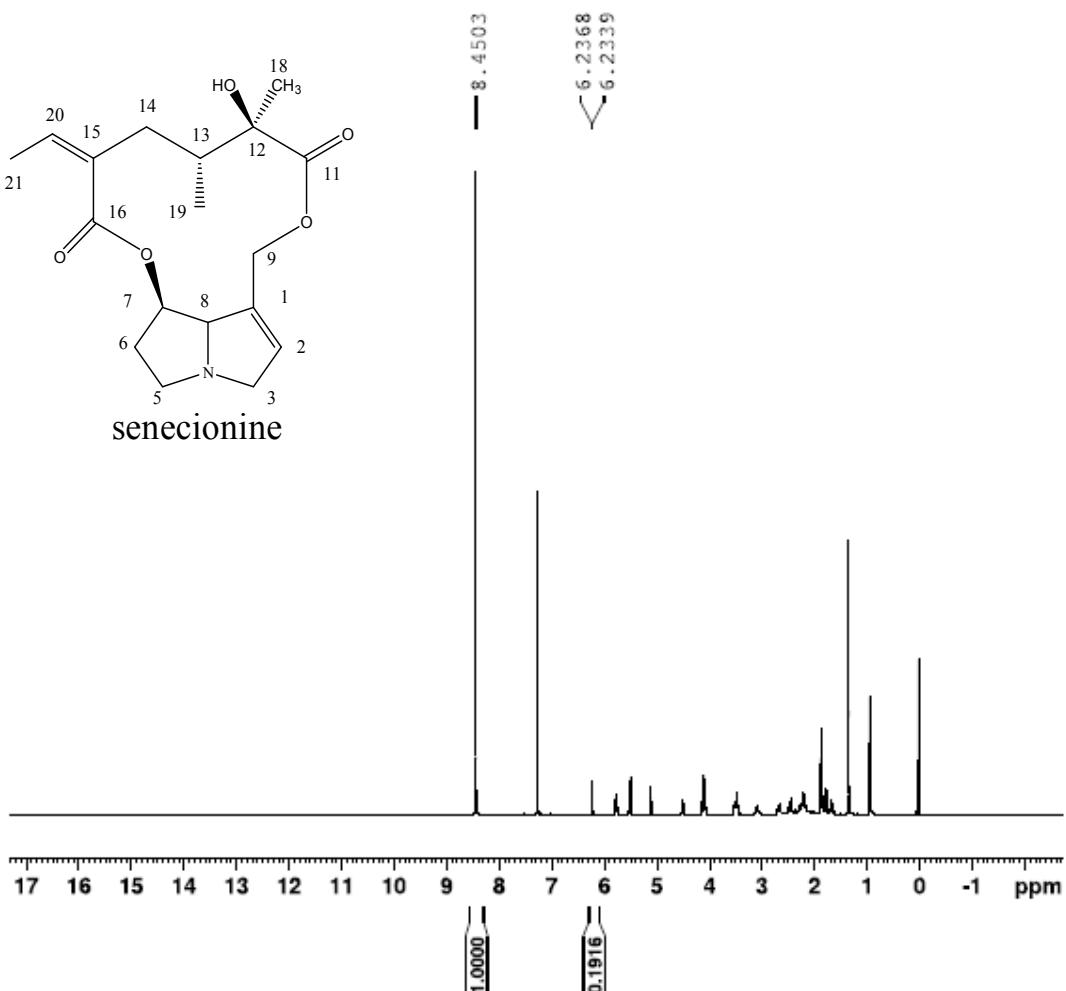


Figure S2 LC-MS chromatogram of senecionine. Senecionine was dissolved in 0.1% formic acid and analyzed on Water UPLC- ZQ2000 MS operating in ESI+ mode. The purity of senecionine (t_R 6.8 min, m/z 336) was calculated about 95% by area normalization method. A small proportion of integerrimine (t_R 6.5 min, m/z 336) was also found.

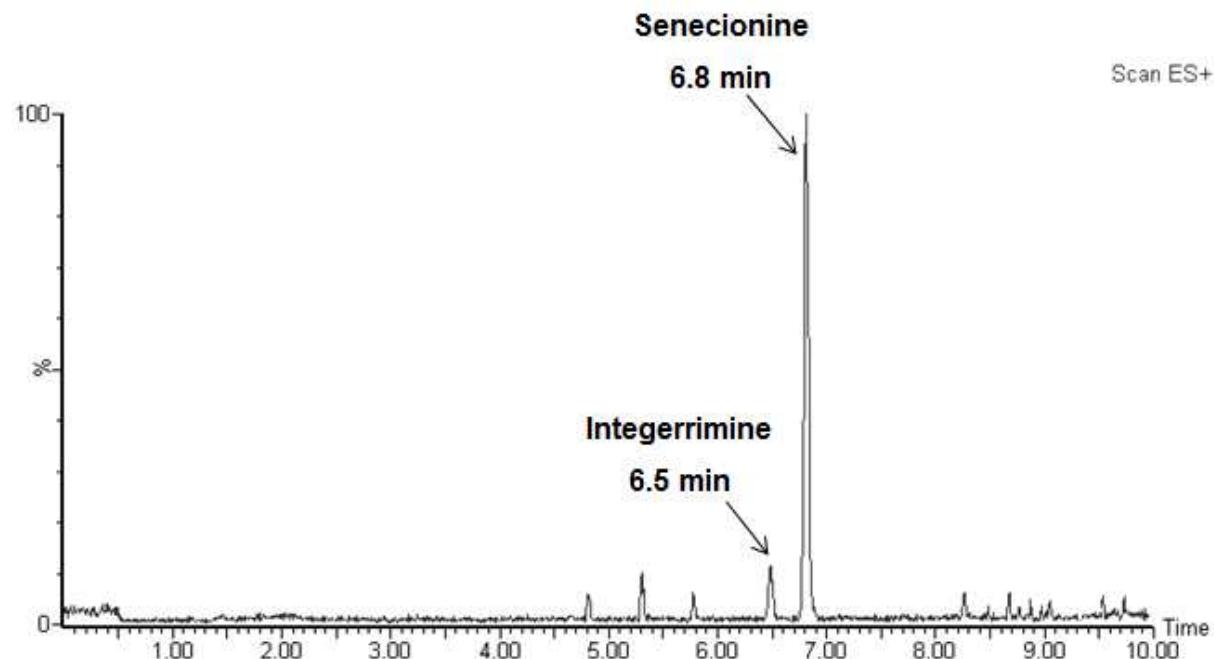
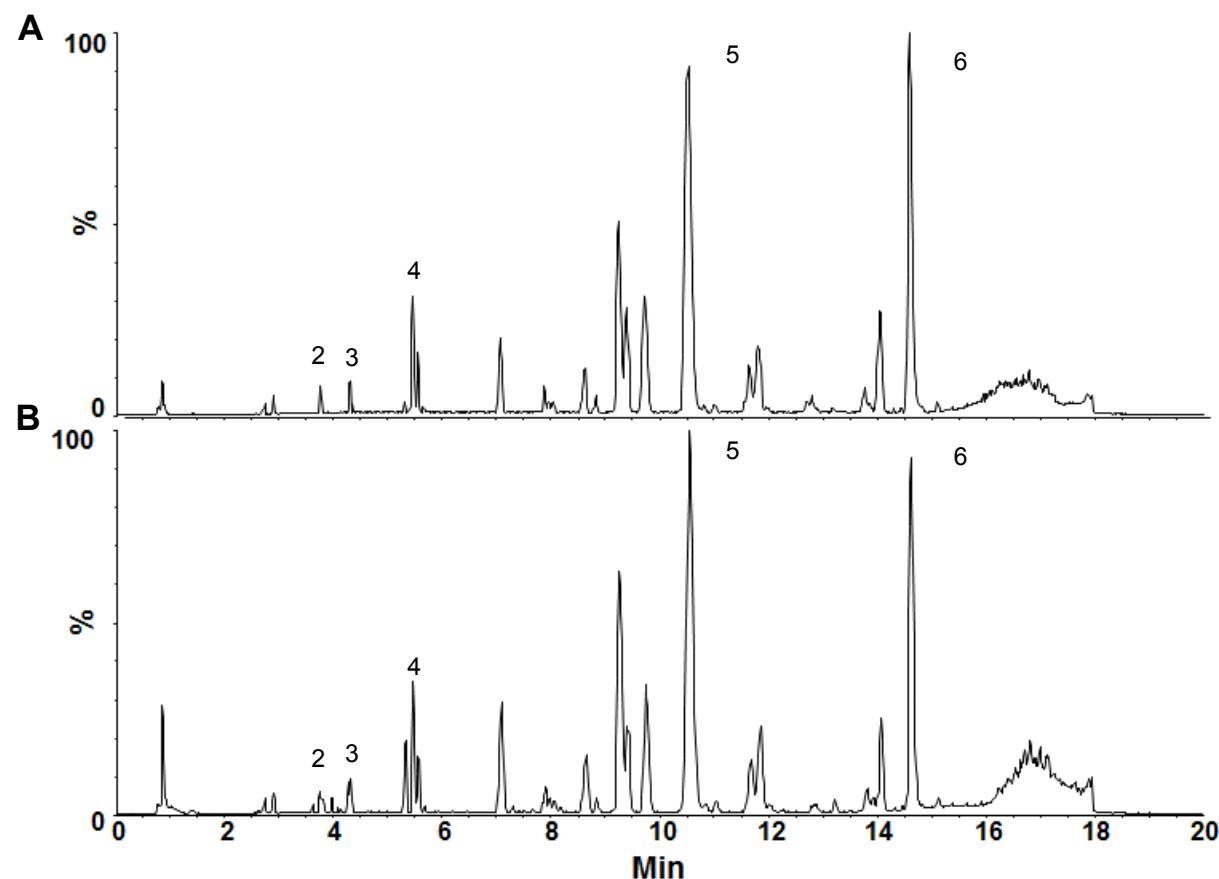


Figure S3 UPLC-MS chromatogram for metabolomic fingerprinting analysis. (A) Serum of control rat in ESI+ mode; (B) Serum of tread rat in ESI+ mode; (C) Serum of control rat in ESI- mode; (D) Serum of treated rat in ESI- mode. (1. TUDCA; 2. TCA; 3. GCA; 4. CA; 5. LPC C16:0; 6. LPC C18:0.)



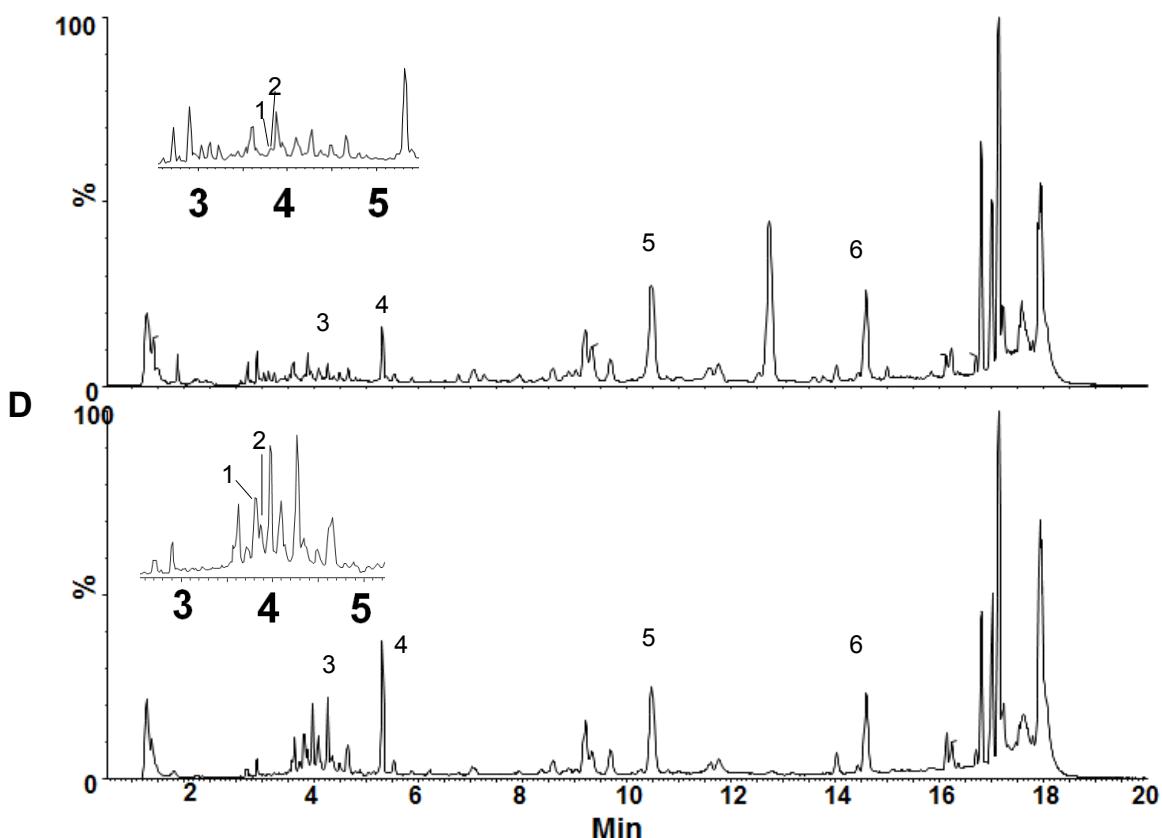
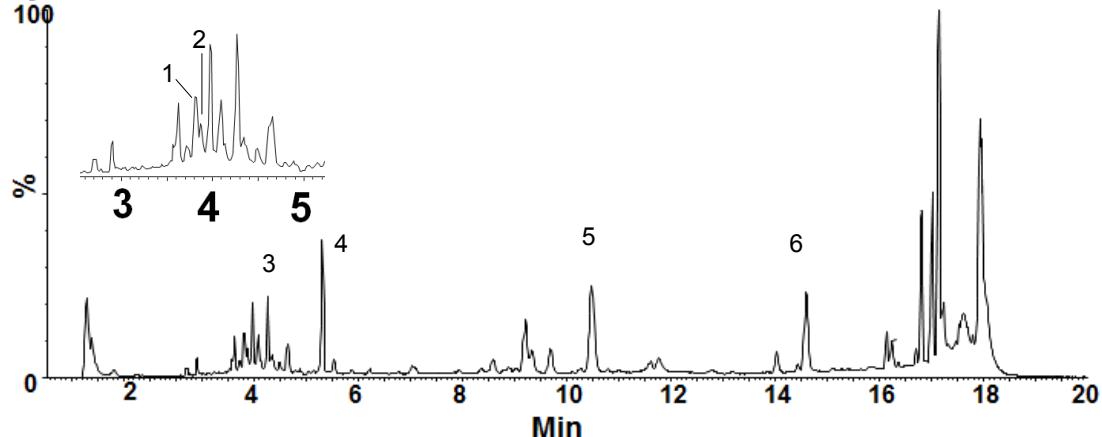
C**D**

Figure S4 UPLC-MS chromatogram for bile acids profiling analysis. Analysis was carried out on Water UPLC-ZQ2000 MS in SIM mode. (1. TUDCA; 2. THDCA; 3. TCA; 4. GUDCA; 5. GCA; 6. CA; 7. TCDCA; 8. TDCA; 9. UDCA; 10. HDCA; 11. GCDCA; 12. GDCA; 13. TLCA; 14. GLCA; 15. CDCA; 16. DCA; 17. LCA.)

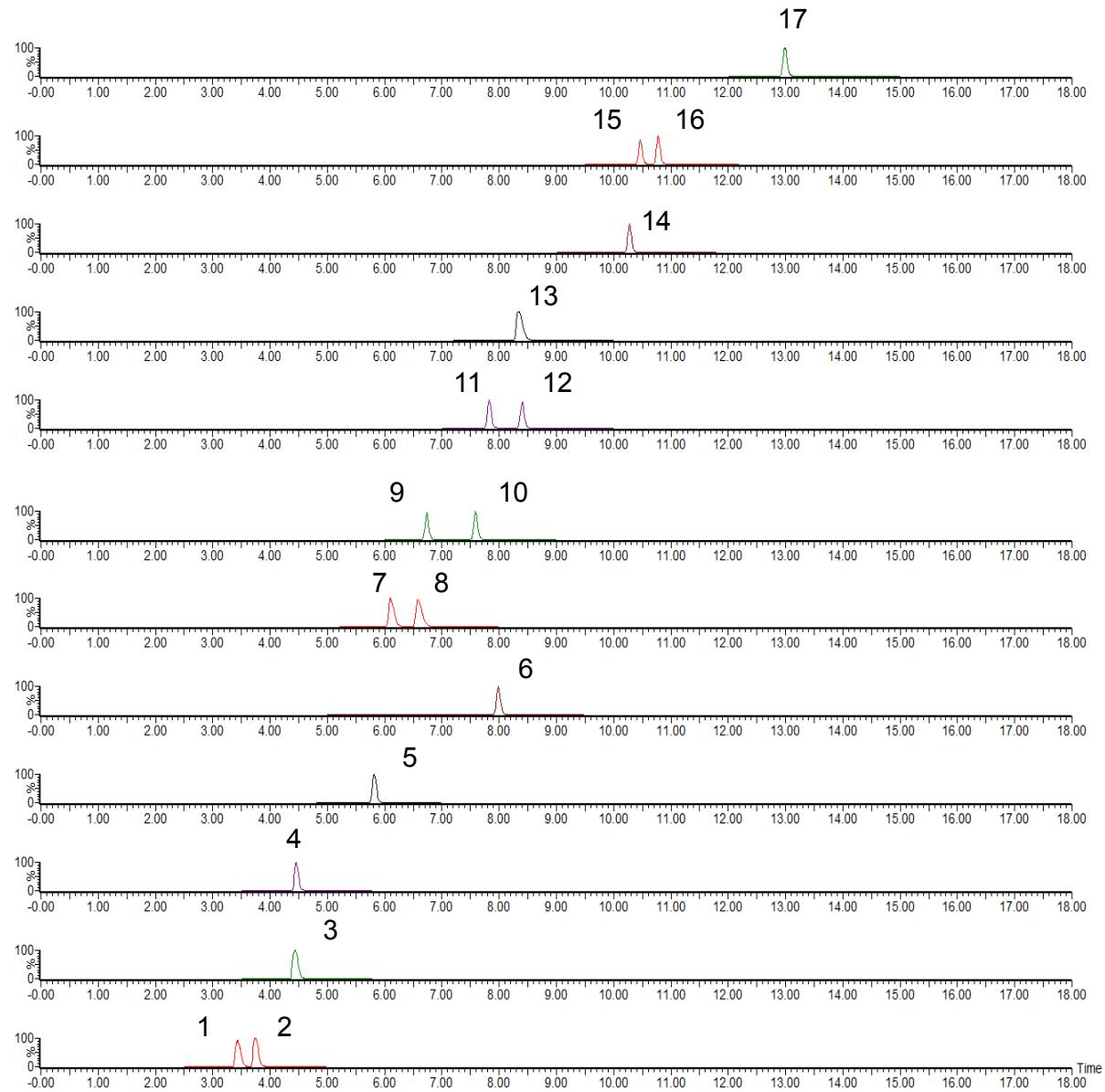


Figure S5 MS/MS fragmentation of LPC C16:0 and C18:0. (A) Spectrum obtained in ESI+ mode; (B) Spectrum obtained in ESI- mode. (1. LPC C18:0 in serum sample; 2. LPC C16:0 in serum sample; 3. LPC C18:0 standard compound; 4. LPC C16:0 standard compound.)

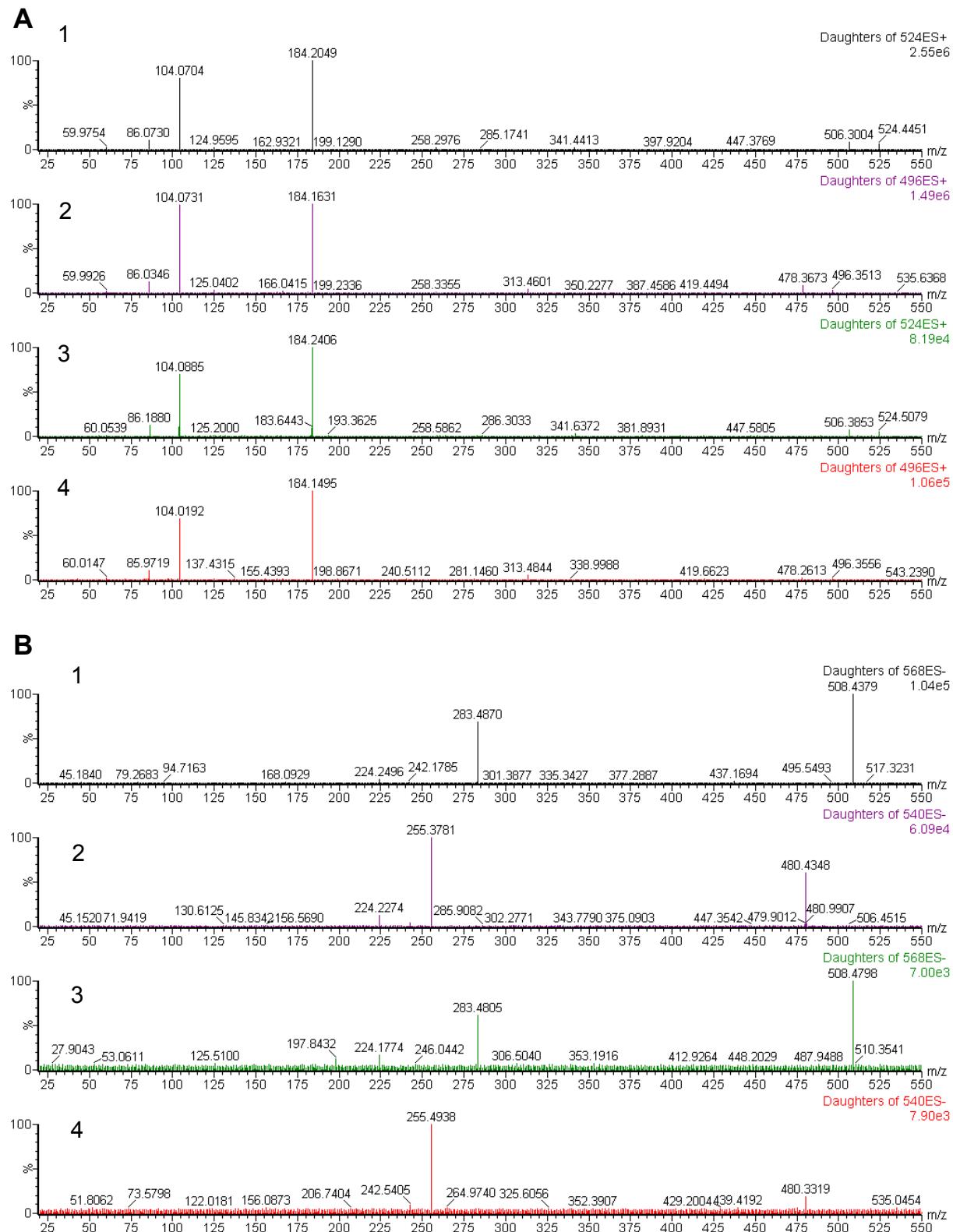


Table S1 Primer pairs used for quantitative RT-PCR.

Gene		Primer Sequence (5'-3')
CYP7A1	F	TACCAAAACCCTCCAGGGAGAT
	R	TGCCAACACAGTGTAGATAGCC
CYP8B1	F	TGGCTGTCGGTTGCCTCTGCCT
	R	GTGTGAACACGTCCCCGTGCTT
BAAT	F	TTTCGTGGTGGGAGAAGATGAT
	R	GCACAGTGGGGAGTAGGGAGGC
NTCP	F	CTCTCTTCCAAC TCAATCCAAG
	R	TGATGATGAGAAGTCCTTCTGC
OAT2	F	CAGCAGAGAGGACTGAACAAAA
	R	GTACGGGTGACTACGAACATGA
OAT3	F	TGGTTGGAGGACCTGTGATTGG
	R	AAGATAACGGTGCTCAGAGAAA
OATP2	F	GGAAAATCCACTGCTGAGGTAG
	R	TCAGACAGGCAGAGCCAGAATG
OATP3	F	CAAGGAGGAGAACACACAGAGAA
	R	TTGGGCAAGAAGGTAAACATAT
OATP4	F	CCAAAACAGATGAGGAGAAGAA
	R	TGAACAGGTAAGTAAAGGAGCC
MRP3	F	ATACCAACACTCCAGACCTCAC
	R	AGATACACCACAGCAGAACACC
BSEP	F	CTGGCTGATGCTTATGGGAGGC
	R	TCTGGTGGAGGAGCTGTTGAT

FXR	F	TCCTCGTCCTATTATTCCAACC
	R	CTCATCCCCTTTATTCTTCCC
RXR□	F	CAAAGACCTGACCTACACCTGC
	R	GACTCCACCTCATTCTCGTTCC
SHP	F	TTGCTAGAGGAACCCAACAGTG
	R	GATAGTGCCTTCAGGTATGCG

Table S2 Pathways significantly interrupted after exposure to senecionine. The enrichment *p* value of the pathway was determined by Fisher's exact test. And the false discover rate of the pathway was also calculated. By setting *p* value < 0.05 and false discovery rate < 0.2, overrepresented changed pathways were identified. "Pathway ID" stands for Pathway identifiers used in KEGG. "Definition" stands for the definition of the Pathway ID. "Fisher-*P* value" stands for the enrichment *p*-value of the PathwayID used Fisher's exact test. "FDR" stands for the false discover rate of the Pathway ID. "Enrichment Score" stands for the Enrichment Score value of the Pathway ID, it equals "-log10(*P* value)".

Pathway ID	Definition	Fisher- <i>P</i> value	FDR	Enrichment Score
<i>down-regulated pathways</i>				
rno00982	Drug metabolism by cytochrome P450	2.03E-11	3.85E-9	10.70
rno04146	Peroxisome	6.91E-11	6.60E-9	10.16
rno00980	Metabolism of xenobiotics by cytochrome P450	9.17E-9	5.84E-7	8.04
rno03320	PPAR signaling pathway	2.34E-8	1.12E-6	7.63
rno00830	Retinol metabolism	4.39E-8	1.68E-6	7.36
rno00260	Glycine, serine and threonine metabolism	2.20E-7	7.02E-6	6.66
rno04976	Bile secretion	6.33E-7	1.73E-5	6.20
rno00330	Arginine and proline metabolism	3.45E-6	8.23E-5	5.46
rno00071	Fatty acid metabolism	8.87E-6	1.88E-4	5.05
rno00280	Valine, leucine and isoleucine degradation	1.16E-5	2.21E-4	4.94
rno00270	Cysteine and methionine metabolism	4.23E-5	7.35E-4	4.37
rno00140	Steroid hormone biosynthesis	4.86E-5	7.74E-4	4.31
rno00650	Butanoate metabolism	1.06E-4	1.56E-3	3.97
rno01040	Biosynthesis of unsaturated fatty acids	1.38E-4	1.88E-3	3.86
rno00380	Tryptophan metabolism	1.68E-4	2.14E-3	3.77

rno00120	Primary bile acid biosynthesis	3.54E-4	4.23E-3	3.45
rno00052	Galactose metabolism	5.32E-4	5.98E-3	3.27
rno00072	Synthesis and degradation of ketone bodies	6.09E-4	6.46E-3	3.22
rno00340	Histidine metabolism	2.59E-3	0.026	2.59
rno00591	Linoleic acid metabolism	3.19E-3	0.031	2.50
rno00250	Alanine, aspartate and glutamate metabolism	3.79E-3	0.032	2.42
rno04080	Neuroactive ligand-receptor interaction	4.21E-3	0.037	2.38
rno02010	ABC transporters	4.72E-3	0.039	2.33
rno00590	Arachidonic acid metabolism	4.93E-3	0.039	2.31
rno00350	Tyrosine metabolism	5.89E-3	0.045	2.23
rno00910	Nitrogen metabolism	6.50E-3	0.048	2.19
rno00640	Propanoate metabolism	7.23E-3	0.051	2.15
rno00310	Lysine degradation	7.92E-3	0.054	2.109
rno04610	Complement and coagulation cascades	9.12E-3	0.058	2.04
rno04920	Adipocytokine signaling pathway	9.12E-3	0.058	2.04
rno04973	Carbohydrate digestion and absorption	0.0126	0.078	1.90
rno00770	Pantothenate and CoA biosynthesis	0.0140	0.083	1.86
rno00500	Starch and sucrose metabolism	0.0161	0.093	1.80
rno00360	Phenylalanine metabolism	0.0186	0.104	1.74
rno00983	Drug metabolism - other enzymes	0.0191	0.104	1.72
rno04020	Calcium signaling pathway	0.0234	0.122	1.63
rno00620	Pyruvate metabolism	0.0239	0.122	1.62
rno05150	Staphylococcus aureus infection	0.0246	0.122	1.61
rno00410	beta-Alanine metabolism	0.0250	0.122	1.60
rno04970	Salivary secretion	0.0256	0.122	1.59
rno00040	Pentose and glucuronate interconversions	0.0288	0.134	1.54

rno04971	Gastric acid secretion	0.0361	0.164	1.44
rno00670	One carbon pool by folate	0.0383	0.170	1.42
rno00062	Fatty acid elongation in mitochondria	0.0405	0.176	1.39
rno00053	Ascorbate and aldarate metabolism	0.0438	0.186	1.36
rno04964	Proximal tubule bicarbonate reclamation	0.0470	0.195	1.33

up-regulated pathways

rno04621	NOD-like receptor signaling pathway	2.13E-7	2.05E-5	6.67
rno04110	Cell cycle	2.17E-7	2.05E-5	6.66
rno05146	Amoebiasis	1.64E-5	1.03E-3	4.79
rno05200	Pathways in cancer	2.18E-5	1.03E-3	4.66
rno04666	Fc gamma R-mediated phagocytosis	3.12E-5	1.18E-3	4.51
rno04810	Regulation of actin cytoskeleton	4.68E-5	1.30E-3	4.33
rno04350	TGF-beta signaling pathway	4.80E-5	1.30E-3	4.32
rno04380	Osteoclast differentiation	8.41E-5	1.99E-3	4.07
rno04510	Focal adhesion	1.14E-4	2.38E-3	3.94
rno04062	Chemokine signaling pathway	1.38E-4	2.56E-3	3.86
rno05142	Chagas disease	1.49E-4	2.56E-3	3.83
rno05145	Toxoplasmosis	2.40E-4	3.77E-3	3.62
rno04670	Leukocyte transendothelial migration	2.60E-4	3.78E-3	3.59
rno05100	Bacterial invasion of epithelial cells	5.04E-4	6.41E-3	3.30
rno05144	Malaria	5.09E-4	6.41E-3	3.29
rno05220	Chronic myeloid leukemia	7.45E-4	8.80E-3	3.13
rno05410	Hypertrophic cardiomyopathy	8.09E-4	8.99E-3	3.09
rno05222	Small cell lung cancer	9.60E-4	9.97E-3	3.02
rno03013	RNA transport	1.01E-3	9.97E-3	2.00
rno03040	Spliceosome	1.05E-3	9.97E-3	2.98

rno05323	Rheumatoid arthritis	1.44E-3	0.012	2.84
rno04620	Toll-like receptor signaling pathway	1.45E-3	0.012	2.84
rno04010	MAPK signaling pathway	1.46E-3	0.012	2.84
rno05140	Leishmaniasis	1.72E-3	0.014	2.76
rno05212	Pancreatic cancer	2.06E-3	0.016	2.69
rno04640	Hematopoietic cell lineage	2.15E-3	0.016	2.67
rno03008	Ribosome biogenesis in eukaryotes	2.33E-3	0.016	2.63
rno04060	Cytokine-cytokine receptor interaction	2.56E-3	0.017	2.59
rno04141	Protein processing in endoplasmic reticulum	3.40E-3	0.022	2.47
rno03450	Non-homologous end-joining	3.61E-3	0.022	2.44
rno05414	Dilated cardiomyopathy	3.64E-3	0.022	2.44
rno04520	Adherens junction	4.06E-3	0.024	2.39
rno05020	Prion diseases	5.80E-3	0.032	2.24
rno05219	Bladder cancer	5.80E-3	0.032	2.24
rno04920	Adipocytokine signaling pathway	6.03E-3	0.032	2.22
rno04210	Apoptosis	7.19E-3	0.032	2.14
rno04512	ECM-receptor interaction	7.42E-3	0.037	2.13
rno00670	One carbon pool by folate	7.47E-3	0.037	2.13
rno04144	Endocytosis	7.92E-3	0.038	2.10
rno05215	Prostate cancer	7.98E-3	0.038	2.10
rno05211	Renal cell carcinoma	0.0138	0.064	1.86
rno04115	p53 signaling pathway	0.0142	0.064	1.85
rno05210	Colorectal cancer	0.0158	0.069	1.80
rno04664	Fc epsilon RI signaling pathway	0.0161	0.069	1.79
rno05412	Arrhythmogenic right ventricular cardiomyopathy	0.0204	0.086	1.69
rno04722	Neurotrophin signaling pathway	0.0248	0.102	1.61

rno00290	Valine, leucine and isoleucine biosynthesis	0.0312	0.125	1.51
rno04530	Tight junction	0.0348	0.137	1.46
rno00520	Amino sugar and nucleotide sugar metabolism	0.0406	0.154	1.39
rno03018	RNA degradation	0.0407	0.154	1.39

Table S3 Serum levels of bile acids ((ng/mL serum). All data were generated by UPLC-MS analysis. Values were expressed as Mean \pm SEM; significant differences between the control group (n=12) and treated group (n=12) are based on the two-tailed unpaired Student's t-test (* $p<0.05$, and ** $p<0.01$).

BA	Con. in control group	Con. in treated group
CA	14917.71 \pm 661.81	17570.29 \pm 715.82
DCA	598.63 \pm 30.92	1074.23 \pm 53.08 *
CDCA	1635.20 \pm 80.99	916.17 \pm 109.15
UDCA	295.25 \pm 16.84	152.37 \pm 17.03
HDCA	2676.97 \pm 225.30	3130.51 \pm 158.55
LCA	7.33 \pm 0.68	6.10 \pm 0.51
GCA	850.57 \pm 62.59	4211.77 \pm 206.42 **
GDCA	97.50 \pm 7.17	270.17 \pm 16.44 *
GCDCA	56.18 \pm 3.68	171.58 \pm 12.57 *
GUDCA	7.22 \pm 0.49	22.53 \pm 1.78 *
GLCA	-	-
TCA	363.59 \pm 18.34	2864.91 \pm 156.02 **
TDCA	50.64 \pm 2.22	409.00 \pm 19.76 **
TCDCA	53.89 \pm 2.61	196.64 \pm 7.39 **
TUDCA	8.24 \pm 0.41	49.69 \pm 1.91 **
THDCA	102.14 \pm 5.72	114.98 \pm 9.64
TLCA	-	-